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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/518,861	03/21/2005	Kenichiro Kosai	042-301	9980

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EXAMINER	
LEAVITT, MARIA GOMEZ	
ART UNIT	PAPER NUMBER
1633	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/518,861	KOSAI ET AL.	
	Examiner	Art Unit	
	MARIA LEAVITT	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 December 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-4,6,7,14-18,21,24,27,30,33,34 and 36* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4,6,7,14-18,21,24,27,30,33,34 and 36* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

1. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
2. Claims 1-4, 6, 7, 14-18, 21, 24, 27, 30, 33, 34 and 36 are pending. Claims 1, 2, 3, 4, 6, 7, 14, 15, 16, 17, 18, 21, 24, 27, 30, 33 have been amended by Applicant's amendment filed on 12-01-2009.
3. Note that claims 21, 24, and 27 were previously withdrawn from consideration because directly or indirectly they depended from cancelled claim 11. Also note that claims 21, 24, and 27 have been amended by Applicant's amendment filed on 12-01-2009 to depend from claim 1 or 2.
4. Accordingly, claims 1-4, 6, 7, 14-18, 21, 24, 27, 30, 33, 34 and 36 are currently under examination to which the following grounds of rejection are applicable.

Response to arguments

Objections withdrawn in response to Applicants' arguments or amendments

Claim Objections

In view of Applicants' amendment of claim 4 to spell out the abbreviations CA, objection to claim 4 has been withdrawn.

In view of Applicants' amendment of claims 15-17, objection to claims 15-17 has been withdrawn.

Rejections maintained in response to Applicants' arguments or amendments

Claim Rejections - 35 USC § 103

Claims 1-4, 6, 7, 14-18, 30, 33, 34 and 36 remain rejected and claims 21, 24, 27 are newly rejected under 35 USC 103 as being unpatentable Vallier et al, (PNAS, 2001, 98:2467-2472) in view Ong et al., US Patent No: 6,777,235, Date or Patent Aug. 17, 2004) and Rybkin et al., (Biol. Chem., 15927-15934, 2003) and further in view of Yamamote et al., (Oncogene 2002, 899-908).

Reply to applicants' arguments as they relate to rejection of Claims 1-4, 6, 7, 14-18, 21, 24, 27, 30, 33, 34 and 36 under 35 USC § 103.

At pages 9-14 of the remarks filed on 12-01-2009, Applicants essentially argue that: 1) the invention solves a problem that was difficult to solve in the prior art and that the Examiner has failed to understand said problem concluding that the invention would be obvious over the prior art of record, 2) in the prior art, the activity of tissue specific promoters in cells stably transfected with a vector comprising a gene encoding the enhanced green fluorescent protein (EGFP) or other fluorescent protein under the control of a cell specific promoter did not reach the level of fluorescence necessary to visualize and isolate the cells by FACS analysis, 3) Applicants have conducted comparative experiments to demonstrate how their invention solves a conventional problem in the art, namely, ES clones of cells at the earliest stages of the cardiac lineage stably transfected with an expression vector encoding the cDNA for EGFP downstream of the Nkx2.5 promoter did not express sufficient levels of EGFP whereas transfection using two DNA constructs by adenoviral vectors into said ES clones expressed sufficient levels of EGFP effective for visualizing and isolating by FACS, 4) the two DNA constructs of the instant invention comprise: a first construct expressing the Cre recombinase under the transcriptional

control of Nkx2.5, and a second construct comprising in sequential order a first a constitutive strong expression promoter, a first LoxP sequence, a drug resistance gene, a second LoxP sequence and a EGFP reporter gene that confers said reporter activity. The second construct constitutively expresses the second EGFP gene under constitutively strong promoter only in a specific cell type after activation of a Nkx2.5 cell-specific promoter in the first construct and 5) " In fact, the technique of the present invention appeared, as the variable research contributing to the stem cell biology, in Molecular Therapy, which is an official journal of "American Society of Gene and Cell Therapy" as one of the most authoritative academic institutes in the field of gene therapy and cell therapy, and, additionally, appeared in the News column in "In this month" (important article in this number) in the same number of the same journal, and, besides, appeared on the cover (meaning the most important article in this number). (See attached reference)". The above arguments have been fully considered but deemed unpersuasive.

Regarding 1)-4), the fact that the instant invention compares results of the weak activity of the Nkx2.5 promoter as used traditionally in stably transfected cells to control expression of EGFP wherein level of fluorescence is insufficient for detection of differentiated earlier stages of cardiac lineage ES cells by FACS and the advantages of employing a Cre-LoxP recombination detection system by incorporating the Cre recombinase gene under the control of the Nkx2.5 promoter to conditionally express Cre which excises the *neo* gene of the first recombinant DNA, which is flanked by a pair of *loxP* sites leading to detectable levels of EGFP by FACS analysis (see pages 38-42 of the specification as filed) is not disputed. However, the examiner disagrees with Applicants upon the nonobviousness of the invention over the combination of Vallier, Ong,

Rybkin and Yamamote for the reasons already of record as set forth at pages 2-6 of the office action filed on 06-01-2009 and the reasons argued in the paragraphs below.

Regarding 5), The examiner notices that the references cited by applicants at page 13 of Applicants' remarks filed on 12-01-2009, have not been provided. Therefore, in the absence of evidences applicants' arguments are merely assertions that cannot take the place of evidence.

At pages 14-16 of the remarks filed on 12-01-2009, Applicants essentially argue that: 1) Vallier' disclosure "relates to the development of a drug-inducible system for expressing a differentiation-induced gene to be introduced only "after a target time" for the promotion of differentiation inducement of an ES cell (see Vallier et al, page 2467, 2nd to 12th line from the bottom), merely uses the CAG (a constitutively activated promoter) and the Cre-loxP system as a means for attaining the object", 2) Vallier does not disclose nor suggest the present invention relating to a method for target cell-specifically enhancing the promoter activity to visualize and isolate the differentiation-induced target cell, 3) Ong et al., neither discloses nor suggests the use of the Cre-loxP system to visualize and isolate an ES cell expressing a tissue-specific promoter with a constitutive strong expression promoter, 4) Ong et al., neither discloses nor suggests the problem with the conventional method for visualizing and isolating a target cell differentiated from an ES cell, 5) Rybkin et al., neither discloses nor suggests the use of Nkx2.5 and Cre-loxP system to visualize and isolate an ES cell expressing a tissue-specific promoter with a constitutive strong expression promoter, 6) Rybkin et al., neither discloses nor suggests the problem with the conventional method for visualizing and isolating a target cell differentiated from an ES cell, 7) Yamamoto involves the *in vivo* use of a Cre-expressing adenovirus which is different from the present invention involving *in vitro* use of adenovirus and 8) Yamamoto et al.,

neither discloses nor suggests the use of a Cre-expressing adenovirus for visualizing and isolating a target cell differentiated from an ES cell. The above arguments have been fully considered but deemed unpersuasive.

Regarding 1), Vallier clearly discloses a second recombinant vector, i.e., the reporter vector (e.g., *pCAG-lox-STOP-EGFP*), comprising a unit of the following genes: a constitutively activated promoter (e.g., CAG) , a first loxP sequence, a drug resistance gene (hygro), a stop sequence, a second loxP and a reporter gene, the expression of which is prevented by tandemly repeated stop-of-transcription sequences flanked by loxP sites. This vector has the same structural limitations of the first recombinant DNA as recited in independent claims 1 and 2. Expression level of EGFP under the control of a constitutively strong expression promoter (e.g., CAG) in the Vallier publication is sufficient to detect cells ES cells expressing EGFP by FACS analysis (page 2468, col. 2, first paragraph). Accordingly, Applicants have not provided probative evidence that there is not sufficient expression of EGFP under the control of a constitutively strong expression promoter to isolate or visualize ES target cells *in vitro* to solve a problem in the prior art . Indeed, the art at the time the invention was made further evidences that ES cells of cardiac lineage stably transfected with a vector encoding EGFP under the transcriptional control of a CMV promoter (e.g., $CMV_{enh.}$ /MLC-2v) can be isolated and visualized by FACS analysis (Muller et al., 2000, FASEB J. 2000, pp.2540-8). Thus the art in an unequivocal manner discloses that level of EGFP expression under the control of a constitutively strong expression promoter is sufficient for FACS analysis. Why then, would expressing EGFP from a constitutively strong expression promoter in an expression vector be insufficient for FACS analysis when the CMV promoter is proven to be a rather good promoter, and used

frequently in adenoviral applications? In relation to using a cell specific promoter, the office has provided motivation for isolating a target ES cell visualized by expression of a fluoresce protein comprising replacing the tamoxifen-dependent Cre inducible recombinase system of Vallier with an alternate cell specific promoter-dependent Cre inducible recombinase system. Furthermore, Applicants are reminded that the motivation for combining the teachings of the prior art may be different from applicants' motivation to make the disclosed compositions. The fact that applicant has recognized another advantage which would flow naturally from following the suggestion of the prior art cannot be the basis for patentability when the differences would otherwise be obvious. See *Ex parte Obiaya*, 227 USPQ 58, 60 (Bd. Pat. App. & Inter. 1985).

Regarding 3), Ong et al., clearly discloses the use of the Cre-loxP system for detection and manipulation of a target eukaryotic gene whose expression is restricted to certain tissues or specialized cell types including the Cre-LoxP recombination system and others (see ¶ [0024] ¶ [0028] of the published application of the published application, for example).

Regarding 5), Rybkin et al., complements the teachings of Vallier and Ong by disclosing gene expression having recombinase recognition sequences on both ends under the control of the Nkx2.5 in culture of mouse cardiomyocytes and used of Adenovirus vectors for *in vitro* transfection of cardiac cell lines.

Regarding 2), 4), 6) and 8), in response to Applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). None of the references has to teach each and every claim limitation. If they did, this would have

been anticipation and not an obviousness-type rejection. Also note that the test for obviousness under 35 U.S.C. 103 requires a highly fact-dependent analysis involving taking the claimed subject matter as a whole and comparing it to the prior art. Therefore, Applicant's argument that Vallier, Ong, Rybkin and Yamamoto neither discloses nor suggests the problem with the conventional method for visualizing and isolating a target cell differentiated from an ES cell is irrelevant.

Regarding 7), the examiner notes that effective *in vivo* therapy using gene transfer with adenovirus vectors makes necessary *in vitro* testing to determine particular conditions effective in the animal models. Accordingly, *in vivo* use of the Cre/loxP system implicitly evidences prior art *in vitro* testing.

Claim Rejections - 35 USC § 112- Second Paragraph

Claims 1-4, 6, 7, 14-18, 21, 24, 27, 30, 33, 34 and 36 remain rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 5 is incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. While all of the technical details of a method need not to be recited, the claims should include enough information to clearly and accurately describe the invention and how it is to be practice. It is not apparent as to under what structural or functional parameters the second recombinant DNA expressing the recombinase under the transcriptional control of a cell-specific promoter activates the first recombinant DNA to express a fluoresce protein gene under the control of a constitutive strong promoter.

Claims 2-4, 6, 7, 14-18, 21, 24, 27, 30, 33, 34 and 36 are indefinite insofar as they depend from claim 1.

The following amendment of claim 1 is suggested by the examiner to point out and distinctly claim the subject matter which the applicant regards as his invention:

An *in vitro* method for selectively isolating or visualizing a target cell differentiated from an embryonic stem cell of human, monkey or mouse, comprising:
a) transferring into an undifferentiated embryonic stem cell an adenovirus vector encoding a first recombinant DNA,
b) inducing differentiation of said undifferentiated embryonic stem cell into which the first recombinant DNA is stably transferred;
c) transferring into said embryonic stem cell during the process of differentiation inducement an adenovirus vector encoding a second recombinant DNA, wherein:
the adenovirus vector in a) comprises in sequential order a first a constitutive strong expression promoter, a first recombinase recognition sequence, a drug resistance gene, a transcription termination codon, a second recombinase recognition sequence and a fluorescence reporter gene that confers said reporter activity, and
the adenovirus vector in b) comprises in sequential order, a cell specific second promoter which is specifically expressed in a target cell differentiated from the embryonic stem cell, a recombinase, a transcription termination codon, and
said given differentiation inducement is effective to induce expression of the second promoter that control expression of the recombinase, and
d) isolating by flow cytometry a target cell differentiated from an embryonic stem cell which is visualized by expression of the fluorescence protein induced by said first promoter.

References made of record in a PTO-892 Form to complete the record

Muller M et al. FASEB J. 2000 pp:2540-8.

Conclusion

Claims 1-4, 6, 7, 14-18, 21, 24, 27, 30, 33, 34 and 36 are rejected.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Leavitt whose telephone number is 571-272-1085. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, Ph.D can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Maria Leavitt/

Maria Leavitt
Primary Examiner, Art Unit 1633